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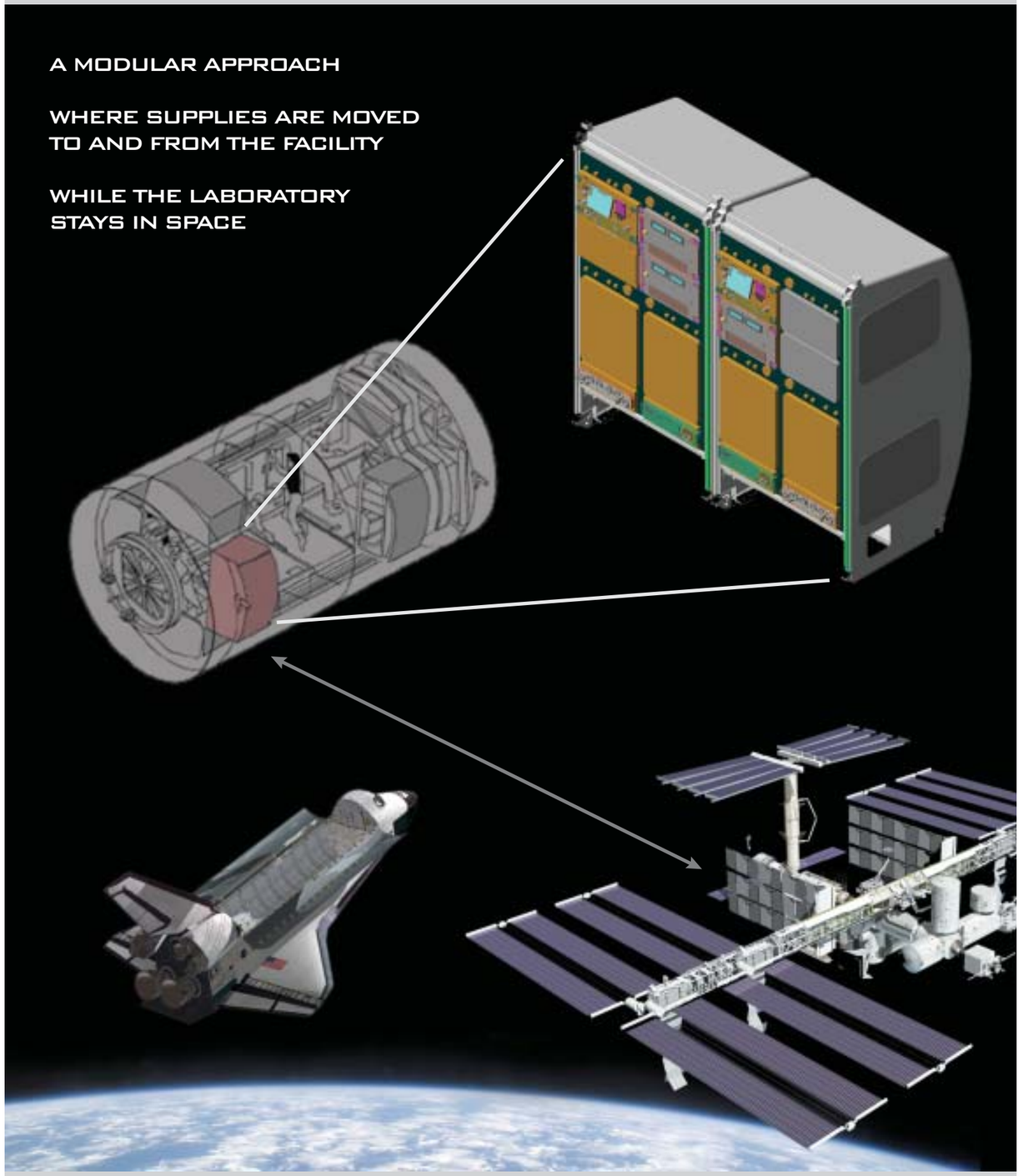
THE BIOTECHNOLOGY FACILITY

A MODULAR APPROACH

WHERE SUPPLIES ARE MOVED
TO AND FROM THE FACILITY

WHILE THE LABORATORY
STAYS IN SPACE



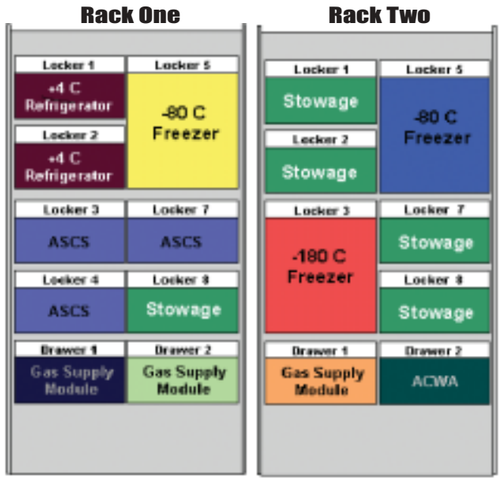
CAPABILITIES OF THE BTF

System Description & Goals

The primary mission of the Cellular Biotechnology Program is to advance microgravity as a tool in basic and applied cell biology. The microgravity environment can be used to study fundamental principles of cell biology and to achieve specific applications such as tissue engineering. The Biotechnology Facility (BTF) will provide a state-of-the-art facility to perform cellular biotechnology research onboard the International Space Station (ISS). The BTF will support continuous operation, which will allow performance of long-duration experiments and will significantly increase the on-orbit science throughput.

With the BTF, dedicated ground support, and a community of investigators, the goals of the Cellular Biotechnology Program at Johnson Space Center are to:

- Support approximately 400 typical investigator experiments during the nominal design life of BTF (10 years)
- Support a steady increase in investigations per year, starting with stationary bioreactor experiments and adding rotating bioreactor experiments at a later date
- Support at least 80% of all new cellular biotechnology investigations selected through the NASA Research Announcement (NRA) process



The above image is the proposed layout of the Biotechnology Facility dual-rack component space.

Enhancements & Increased Scientific Return

The BTF will employ several methods to increase scientific return over the Cellular Biotechnology Operations Support System (CBOSS), which was an interim platform for cellular biotechnology research on ISS.

- Automated experiment operations - to reduce crew time requirements and to standardize experiment procedures
- Modular components - to allow sequential and continuous experiment operations without cross-contamination
- Increased cold storage capability (+4˚C, -80˚C, -180˚C)
  - Storage of frozen cell culture inoculum - to allow sequential investigations
  - Storage of post-experiment samples - for return of high quality samples
- Increased number of cell cultures per investigation, with replicates - to provide sufficient number of samples for data analysis and publication of results in peer-reviewed scientific journals

	Days of on-Orbit Operations Per 120 day Increment	Number of Stationary Cultures Per Increment	Crew Hours Required (Per Increment)	Cultures Per Crew Hour
Current Capability of CBOSS	14 days	32	26	1.2
Future Capability BTF	110 days	324	55	5.9

= **10X Increase** IN TOTAL SCIENCE THROUGHPUT

5X Decrease IN CREW TIME PER CULTURE

These numbers are based on the July 2003 ASCS Preliminary Design Review level of design maturity and subject to change

Core Functions

The BTF will be a complete research laboratory facility that is equipped with the resources necessary to conduct cellular biotechnology experiments in the microgravity environment on the International Space Station. The BTF components provide four core functions:

Cell Culture Incubation
The BTF will provide a controlled environment for the cultivation of cells into three-dimensional tissues. These cell and tissue cultures, as well as the conditioned media, will be the primary source of sample material available to investigators for analysis.

Experiment Support
The BTF will provide the materials and hardware required to support cell and tissue culture experiments.

- Production of cell culture media
- Supply of tissue culture grade gas
- Cold storage
  - +4˚C: storage of culture media and other supplies, post-experiment samples
  - -80˚C: storage of media supplements, post-experiment samples
  - -180˚C: storage of cell culture inoculum
- Ambient storage for experiment supplies

Experiment Assessment
The BTF will provide capabilities to assess cell and tissue culture experiments. Capabilities include sensors in BTF components and use of a portable clinical blood gas analyzer.

Command and Control/Data Handling
The BTF will provide the command and control functions required to monitor and control the operation of BTF racks, to monitor the health and status of the BTF sub-rack components, and to partition resources to ensure that the maximum science return is achieved. The BTF will also provide experiment command and control/data handling functions. These will include telemetry downlink and real-time command uplink from BTF Operations Support Facilities.

FIELDS OF INVESTIGATION

Major Investigation Areas

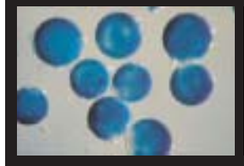
3-D Tumor Modeling	Cellular Metabolism	Pharmaceutical Studies
Angiogenesis	Cellular Physiology	Proteomics
Apoptosis	Differentiation	Radiation Modeling
Biomaterials	Genomics	Signal Transduction
Biosensors	Membrane Dynamics	Tissue Engineering
Biosentinels	Microencapsulation	Tissue Morphogenesis
Cellular Countermeasures	Models for Disease	Tissue Transplantation
Cellular Locomotion	Molecular Biology	Toxicology

Some Typical Goals for Microgravity Experiments

- Compare activation and/or deactivation of genes
- Examine alterations in cell motility
- Examine changes in cellular structure
- Examine changes to signal transduction pathways
- Examine alterations to enzyme-mediated chemical activity
- Observe apoptotic changes
- Construct complex functional tissues
- Compare types and quantity of protein production

Chung, Leland: Tissue Response to Androgen Therapy and Microgravity *(BDS-05, STS-107)*

Becker, Jeanne L.: Evaluation of Ovarian Tumor Cell Growth and Gene Expression *(CBOSS-1, ISS Expedition 3)*



Li, Albert: Assessment of Human Liver Function in Microgravity *(StelSys, ISS Expedition 5)*

Hammond, Timothy G.: Renal Cell Differentiation and Hormone Production from Human Renal Cortical Cells *(CBOSS-2, ISS Expedition 4)*

Jessup, J. Milburn: Use of NASA Bioreactor to Study Cell Cycle Regulation Mechanisms of Colon Carcinoma Metastasis in Microgravity *(CBOSS-1, ISS Expedition 3)*

Morrison, Dennis R.: Microencapsulation Electrostatic Processing System II *(MEPS-II, ISS Expedition 5)*

Application of Findings

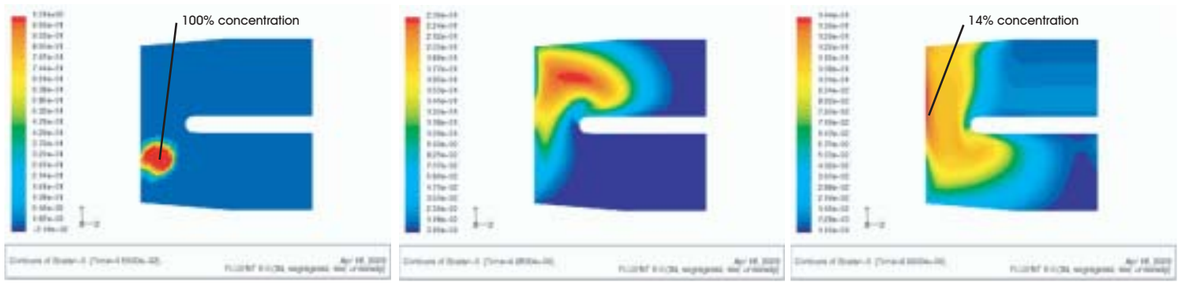
Microgravity can be used as a powerful research tool. Cellular responses to the stress of exposure to microgravity can provide insight into basic cellular mechanisms. The microgravity environment can also be used to facilitate construction of functional tissues that either can not be assembled in 1G or that are difficult to grow on Earth. Knowledge gained from cellular biotechnology microgravity experiments can be applied to disease fighting efforts on Earth and aid in the genesis of potential cellular countermeasures for human space flight.

ENGINEERING DATA

Computational Fluid Dynamics Analysis

Mixing Characteristics:

- A mixing procedure is necessary for the stationary culture vessels to distribute inoculum, fresh media, or other treatments uniformly throughout the cell culture vessel volume.
- Mixing will also provide an even distribution of nutrients throughout the cell culture vessel, which is essential to promote cell growth and aggregate formation.
- A kneading-like mixing protocol that transfers the cell culture media from one side of the culture vessel to the other has been proposed for use in the Automated Stationary Culture System (ASCS).
  - This protocol has been modeled using FLUENT™, a computational fluid dynamics tool.
- The mixing process will produce low mechanical stress levels and a laminar flow to avoid damage to the shear sensitive cells.
- The mixing protocol will use a low frequency of oscillation (0.1 Hz) and a 1.5 ml volume displacement to produce a 0.0024 dynes/cm^2 maximum shear stress, which is 60-fold less than the critical 0.15 dynes/cm^2 level which harms cells.
- Preliminary CFD analysis has demonstrated that this protocol will result in effective mixing within minutes.



START *TIME = 0 SECONDS*

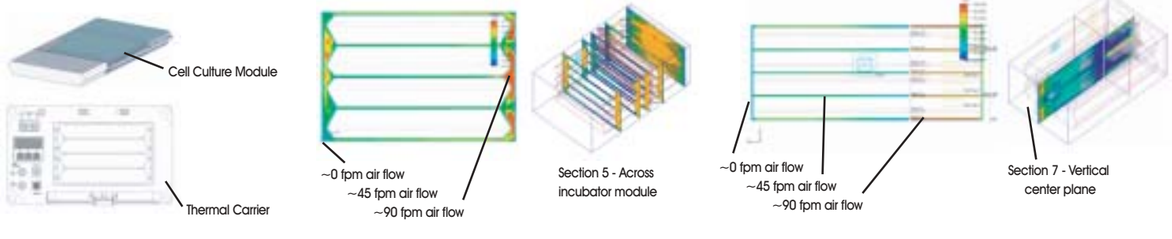
AFTER 1.5 CYCLES *TIME = 5 SECONDS*

AFTER 3 CYCLES *TIME = 10 SECONDS*

These images show the results of a fluid dynamics analysis conducted using FLUENT™ software to simulate the proposed method of mixing within the culture vessels. The simulation tracks mixing of relative oxygen concentration from 100% to 14% over three cycles. Note that the color scale is different for each figure. *This preliminary data is based on saturated oxygen fluid modeling. Additional analysis is required to evaluate cells, cell aggregates, and media agent mixing.*

ASCS Thermal Carrier Heat Rejection Capability:

- The ASCS Thermal Carrier will provide a +4ºC environment to house the Cell Culture Modules. This allows experiment supplies located in the Cell Culture Module to be refrigerated, while cells are cultured at the appropriate conditions inside the "micro-incubator" section of the module. At the end of an experiment, the micro-incubators are turned off so that the fixed cultures are stored at +4ºC until they can be transferred to long-term cold storage.
- Sufficient airflow around the Cell Culture Modules is required for the ASCS thermal carrier to maintain its +4ºC environment. The packaging of the Cell Culture Modules will affect this airflow.
- Computational Fluid Dynamics (CFD) models were developed for two layout concepts using Icepak™ to analyze the airflow around Cell Culture Modules within the Thermal Carrier.
- The analysis showed that the flat profile Cell Culture Module with beveled ends (shown in the CFD plots) along with a baffle allowed for an even distribution flow within the cold volume at a rate of 3.4 cfm.
- The even flow distribution along with the heat lift capacity of the thermal carrier will ensure a uniform +4ºC environment throughout the chamber.
- A complete thermal model, incorporating the airflow model and the power dissipating components within the cell culture modules, will be developed to verify the thermal performance of the ASCS.



CFD velocity speed contours indicate uniform air flow across all surfaces of the module. This will result in sufficient cooling within the Thermal Carrier.

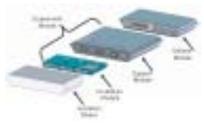
COMPONENTS OF THE BTF

Biotechnology Facility Components

The BTF will consist of two Expedite the Processing of Experiments to the Space Station (EXPRESS) racks containing the sub-rack elements required to conduct cellular biotechnology research. Experiment hardware will feature a "high through-put" cell and tissue culture system and several pieces of experiment-support hardware. The Automated Stationary Culture System (ASCS) is designed to accommodate multiple cultures and multiple investigations simultaneously. Additionally, the ASCS will allow end-to-end increment operation on ISS. Experiment support hardware will include: a water purification and media mixing system to produce cell culture media on-orbit (ACWA), a gas supply module (GSM), and dedicated cold storage facilities.

Automated Stationary Culture System (ASCS)

- Two major components: Thermal Carrier and Cell Culture Modules
- Thermal Carrier provides +4˚C storage for experiment supplies and preserved cultures
- Cell Culture Modules contain an insulated micro-incubator to provide appropriate environment for incubation of cell and tissue cultures
- Four Cell Culture Modules per unit, 3 cell culture vessels per module
- Cell culture vessel: 10 ml bag
- Format of samples: fixed cell cultures and media samples
- Incubation temperature set point range: +25˚C - 45˚C ± 1˚C
- CO_2 set point range: 0 - 10% ± 1%
- Sample fixative storage temperature range: +4˚C - 30˚C ± 1˚C
- Sensor capabilities: pH, CO_2, temperature
- Automated capabilities: media exchanges, low-shear mixing of culture vessel contents, pH monitoring, media sampling, culture wash and fixation
- Manual capabilities: sample port on each cell culture vessel

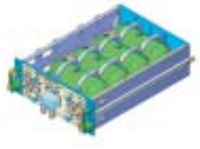


ASCS Cell Culture Module

- Automated, semi-autonomous, self-contained cell culture incubator
- Incubation Module – micro-incubator that contains 3 culture vessels, surrounded by insulation sleeve
- Support Module – contains fluid reservoirs, media samples, sensors, solidstate memory device with pre-programmed experiment operations table
- Utilities Module – reuseable; provides electrical and mechanical interfaces, module controller
- Experiment Module (Support Module + Incubation Module) – pre-assembled and sterilized prior to flight; can be refurbished on the ground
- Modular design allows sequential experiments to be performed without cross-contamination

Automated Culture Water Assembly (ACWA)

- Process water from any ISS potable water source to cell culture grade water
- Mix water, liquid media concentrate, and pre-thawed media supplements to produce cell culture media
- Dispense the produced media into the container(s) required by the BTF cell culture hardware
- Prevent cross-contamination from one media production to the next
- Allow on-orbit filter change-out for extended duration operation



Gas Supply Module (GSM)

- Provide gas for BTF cell culture hardware
- On-orbit tissue-culture grade gas with between 0 and 10% CO_2 that has been pre-mixed on the ground
- Two gas output ports capable of simultaneous operation
- Flow rate delivery of a maximum of 1000 standard cubic centimeters per minute (SCCM)
- Quick disconnect regulated gas pressure of 40 ± 7 psig
- Front panel displays for the storage pressure and the delivery pressure

Cold Stowage (BTR, GLACIER, ARCTIC)

- Dedicated cold storage facilities for refrigeration and cryogenic preservation
- +4˚C, -80˚C, -180˚C
- Internal and external temperature sensing capabilities

BTF Operations Support Facilities

- BTF real-time operations support will be coordinated from two dedicated workstations in the Telescience Support Center (TSC) at Johnson Space Center's Mission Control Center (MCC)
  - Ground to ground voice loops for communicating with the Payload Operations Integration Center (POIC) at Marshall Space Flight Center
  - ISS space to ground voice loops for monitoring crew communications
  - ISS payload downlink telemetry
  - ISS video downlink
  - Ground commanding
- Alternate site for operations and experiment support established at Wyle Laboratories in Houston, TX
- Investigators may monitor appropriate downlink data from a remote site through a password protected website

DEVELOPMENT STRATEGY

Concepts Proven In Development Units

In the Preliminary Design phase, tests are performed at the system, subsystem, or component level to show that a specific design approach is acceptable to meet the functional and/or performance requirements of the hardware, firmware, and software. Development units (proof of concept models and test beds) are composed of hardware and software that perform the basic functions of an engineering unit, but typically differ in form and fit.

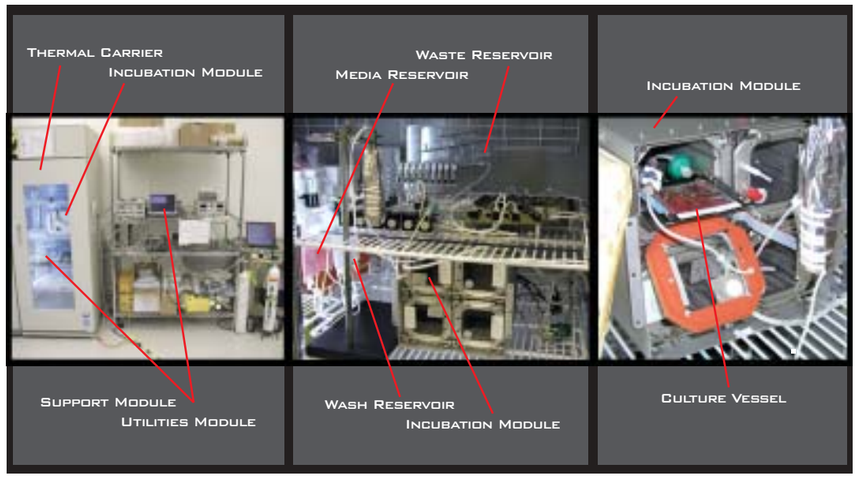
Concepts Validated in ASCS Model and Breadboards

- Incubation chamber housed in a +4˚C volume maintains appropriate environment to support viable cell cultures (37˚C, 5% CO_2)
  - Robust, fast growing cell lines
  - Sensitive cell lines
  - Cell lines that utilize microcarrier beads
- Automation of Stationary Cell Culture Operations
  - Rapid thaw of frozen inoculum
  - Media exchanges
  - Mixing of culture vessel contents
  - Collection of media samples
  - Cell culture wash and fixation
- Short-term preservation of fixed cultures at +4˚C

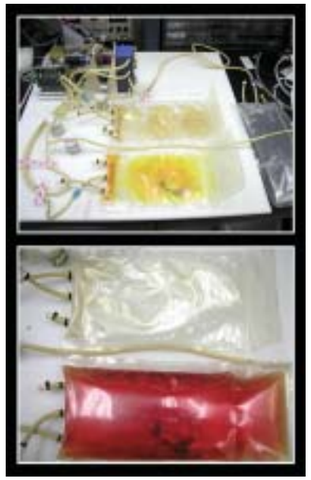
ACWA Test Beds

- Water purification test bed
  - Ion exchange resin and activated carbon to remove biocides present in ISS water sources
  - Biological filters to remove endotoxins and bacteria
  - Biocompatibility testing of purified water
  - Gene array analysis on cells cultured in media produced with ACWA-purified water
- Media mixing test bed
  - Lab supplied water mixed with concentrated media and supplements
  - Bubble removal testing
  - Cells cultured in ACWA-mixed media evaluated for growth, morphology and nutrient utilization
- Complete ACWA test bed
  - Purify water and mix with concentrated media and supplements
  - Cells cultured in ACWA-prepared media evaluated for growth, morphology and nutrient utilization

ASCS Proof of Concept Model



ACWA Media Mixing Test Bed



Engineering Unit Development

Upon completion of the preliminary design phase and subassembly 'proof of concept' testing, development of Engineering Units of the three primary subrack components - ASCS, ACWA and GSM - will be initiated. The Engineering Unit hardware, firmware and software components will be equivalent to the Qualification & Flight Units. Fabrication of the Engineering Units provides the means to resolve engineering, manufacturing and assembly challenges, resulting in finalized design and manufacturing drawings. Testing of the Engineering Units allows for end-to-end science verification of the individually proven concepts, and evaluation of qualification and certification requirements (vibration, acoustic, EMI, thermal cycle, etc.) prior to fabrication of the Qualification & Flight Units. Upon completion of testing, the Engineering Units will be utilized for training and sustaining engineering purposes.

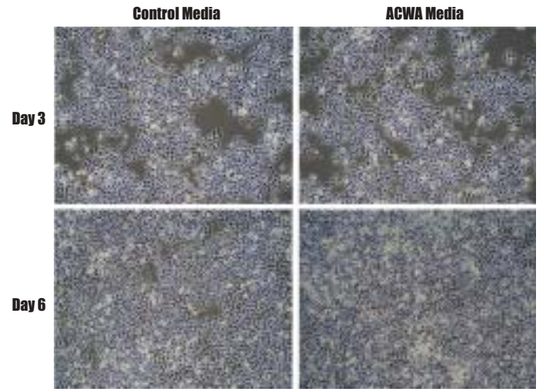
Flight Hardware Development

Upon completion of the critical design phase and appropriate Engineering Unit testing, development of the Qualification and Flight Units of the three primary subrack components - ASCS, ACWA, and GSM - will be initiated. The Qualification Units hardware, firmware and software will be identical to the Flight Units in every aspect (form, fit and function as well as in manufacturing processes, parts selection and quality control). The Qualification Units will be used for verification and certification of all environmental requirements, as well as performance requirements as needed. Testing of the Qualification Units will verify that the items will perform in the launch and operational environments onboard ISS. Upon completion of testing, the Qualification Units can be refurbished for use as Flight Units. The Flight Units are manufactured under strict quality control, and complete records of manufacturing, testing, and transportation are maintained. A series of acceptance tests will be performed on each Flight Unit as part of the certification process. Upon completion of all testing, the Flight Units will be delivered to ISS.

CELL GROWTH IN EARLY ENGINEERING UNITS

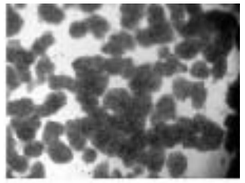
Cells Cultured in Media Prepared with ACWA

- Initial tests in the ACWA demonstrated that cellular growth, morphology and glucose utilization were similar between cells cultured in media prepared with ACWA and in control media.
- The ACWA mixing test bed was utilized to prepare GTSF-2 media and alpha-MEM media by mixing cell culture grade water with 3X or 10X liquid media concentrate and media supplements.
- MIP-101 human colorectal carcinoma cells and WI-38 human lung fibroblasts were cultured in the ACWA prepared media and in lab-prepared control media for six days.

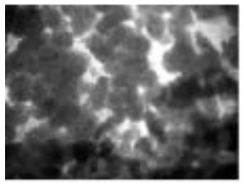


Cells Cultured in ASCS Model

24 hours (10X Objective)



5 days (4X Objective)

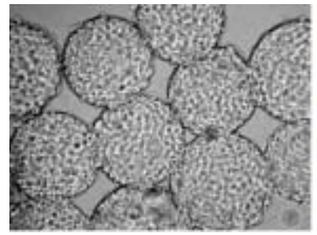


7 days (4X Objective)

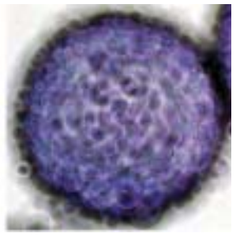
MTT Test (20X Objective)

- Initial tests in the ASCS Proof of Concept Model demonstrated that an insulated incubation chamber housed at +4˚C will support viable cell cultures.
- MIP-101 human colorectal carcinoma cells were successfully cultured in the ASCS for seven days. Cellular growth, morphology and nutrient utilization were similar to control cultures housed in a standard laboratory incubator.
- Within 24 hours of inoculation, MIP-101 cells formed three-dimensional aggregates. As the cells proliferated, the aggregates increased in size and number.
- Following the test, aggregates were exposed to MTT reagent to confirm cell viability. Metabolically active cells appeared purple.
- In additional tests, EMS-3 Rauscher murine erythroleukemia cells were successfully cultured in the ASCS model.

Cells on Microcarrier Beads in ASCS Model



7 days (20X Objective)



Fixed cells with Hematoxylin Stain

- Subsequent tests in the ASCS Proof of Concept Model demonstrated that the key concepts required to perform a complete cell culture experiment in the ASCS can be successfully achieved.
  - Rapid thaw of frozen inoculum
  - Dilution and removal of cryopreservation agent
  - Culture incubation (37˚C, 5% CO_2)
  - Automated media exchanges
  - Automated collection of media samples
  - Automated wash and fixation of cell cultures
  - Short-term storage of fixed cultures at +4˚C
- MIP-101 human colorectal carcinoma cells were cultured with microcarrier beads in the ASCS for seven days. Cellular growth, morphology and nutrient utilization were similar to control cultures housed in a standard laboratory incubator.
- Cells were washed with PBS and fixed with formalin.
- Following the test, fixed cells were stained with hematoxylin to confirm preservation of cell morphology.